CLAIMS

What is claimed is:

1. A crystalline GR polypeptide complex comprising an expanded binding pocket.

2. The polypeptide complex of claim 1, wherein an AF2 helix is located in an active position, and where atoms in residues Met560, Met639, Gln642, Cys643, Met646, and Tyr735 have shifted from their positions in a GR/Dex structure, characterized by the atomic structural coordinates of Table 3, by one of a heavy-atom RMS deviation of at least about 0.50 angstroms and by a backbone heavy-atom RMS deviation of at least about 0.35 angstroms.

3. The polypeptide complex of claim 1, wherein an AF2 helix is located in an active position, and wherein atoms in residues Met560, Met639, Gln642, Cys643, Met646, and Tyr735 have shifted from their positions in a GR/Dex structure, characterized by the atomic structural coordinates of Table 3, so as to increase the volume of the main binding pocket by at least about 5%, compared with a GR/Dex structure characterized by the atomic structural coordiates of Table 3.

4. The polypeptide complex of claim 1, wherein an AF2 helix is located in an active position, and wherein atoms in and around a ligand binding site have shifted from their positions in a GR/Dex structure, characterized by the atomic structural coordinates of Table 3, so as to accommodate, without atomic overlap, a steroidal ligand with 17-α substituents comprising 2-20 atoms.

5. The polypeptide complex of claim 1, wherein an AF2 helix is located in an active position, and wherein atoms in and around a ligand binding site have shifted from their positions in a GR/Dex structure,

characterized by the atomic coordinates of Table 3, so as to accommodate, without atomic overlap, a non-steroidal ligand.

6. The polypeptide complex of claim 5, wherein the non-steroidal ligand is selected from the group consisting of benzoxazin-1-one and A-222977.

7. The polypeptide complex of claim 1, wherein an AF2 helix is located in an active position, and wherein atoms in and around a ligand binding site have shifted from their positions in a GR/Dex structure, characterized by the atomic coordinates of Table 3, such that fluticasone propionate can be docked into a binding site with a favorable binding energy and wherein all atoms in the polypeptide are held fixed.

8. The polypeptide complex of claim 1, wherein an AF2 helix is located in an active position, and wherein atoms in and around a ligand binding site have shifted from their positions in a GR/Dex structure, characterized by the atomic coordinates of Table 3, such that a non-steroidal GR ligand can be docked into the binding site with a favorable binding energy, as computed with molecular modeling software and wherein all atoms in the polypeptide are held fixed.

9. The polypeptide complex of claim 8, wherein the non-steroidal ligand is selected from the group consisting of benzoxazin-1-one and A-222977.

10. The polypeptide complex of claim 1, further comprising fluticasone propionate and a co-activator peptide.

11. The polypeptide complex of claim 10, wherein the crystalline form comprises lattice constants of a = b = 127.656 Å, c = 87.725 Å, α = 90°, β = 90°, γ = 120°.

12. The polypeptide complex of claim 10, wherein the co-activator peptide is a TIF2 peptide.

13. The polypeptide complex of claim 12, wherein the TIF2 peptide comprises the sequence of SEQ ID NO: 9.

14. The polypeptide complex of claim 10, wherein the complex comprises a hexagonal crystalline form.

15. The polypeptide complex of claim 10, wherein the crystalline form has a space group of $P6_1$.

16. The polypeptide complex of claim 10, wherein the GR polypeptide comprises a GRα ligand binding domain.

17. The polypeptide complex of claim 16, wherein the GRα polypeptide has the amino acid sequence shown in any one of SEQ ID NOs: 6 or 8.

18. The polypeptide complex of claim 16, further characterized by the atomic structure coordinates shown in Table 2.

19. The polypeptide complex of claim 16, wherein the crystalline form comprises two GRα ligand binding domain polypeptides in the asymmetric unit.

20. The polypeptide complex of claim 16, wherein the complex is such that the three-dimensional structure of the crystallized GRα ligand binding domain polypeptide can be determined to a resolution of about 3.0 Å or better.

21. The polypeptide complex of claim 10, wherein the complex comprises one or more atoms having a molecular weight of 40 grams/mol or greater.

22. A method for determining the three-dimensional structure of a crystallized GR polypeptide complex comprising an expanded binding pocket to a resolution of about 3.0 Å or better, the method comprising:

(a) crystallizing a GR ligand binding domain polypeptide; and

(b) analyzing the GR ligand binding domain polypeptide to determine the three-dimensional structure of the crystallized GR ligand binding domain polypeptide, whereby the three-dimensional structure of a crystallized GR polypeptide complex comprising an expanded binding pocket is determined to a resolution of about 3.0 Å or better.

23. The method of claim 22, wherein the polypeptide complex further comprises fluticasone propionate and a co-activtor peptide.

24. The method of claim 23, wherein the crystallization is accomplished by the hanging drop method, and wherein the GR ligand binding domain, the fluticasone propionate and the co-activator peptide are mixed with a reservoir solution.

25. The method of claim 24, wherein the reservoir solution comprises 60mM bis-Tris-propane, pH 7.5-8.5, and 1.5-1.7 M magnesium sulfate.

26. The method of claim 23, wherein the co-activator peptide is a TIF2 peptide.

27. The method of claim 26, wherein the TIF2 peptide comprises the sequence of SEQ ID NO: 9.

28. The method of claim 22, wherein the GR ligand binding domain comprises one of SEQ ID NO: 6 and SEQ ID NO: 8.

29. The method of claim 22, wherein the analyzing is by X-ray diffraction.

30. A method of generating a crystallized GR polypeptide complex comprising an expanded binding pocket and a ligand known or suspected to be unable to associate with a known GR structure, the method comprising:

(a) providing a solution comprising a GR polypeptide and a ligand known or suspected to be unable to associate with a known GR structure; and

(b) crystallizing the GR ligand binding domain polypeptide using the hanging drop method, whereby a crystallized GR polypeptide complex comprising an expanded binding pocket and a ligand known or suspected to be unable to associate with a known GR structure is generated.

31. The method of claim 30, wherein the polypeptide complex further comprises fluticasone propionate and a co-activator peptide.

32. The method of claim 30, wherein the solution comprises 475 mM ammonium acetate, 25 mM NaCl, 50 mM Tris, pH 8.0, 10% glycerol, 10 mM dithiothreitol (DTT), 0.5mM EDTA and 0.05% β-octyl-glucoside.

33. The method of claim 30, wherein a crystallization reservoir solution comprises 60mM bis-Tris-propane, pH 7.5-8.5, and 1.5-1.7 M magnesium sulfate.

34. The method of claim 31, wherein the co-activator peptide is a TIF2 peptide.

35. The method of claim 34, wherein the TIF2 peptide comprises the sequence of SEQ ID NO: 9.

36. The method of claim 30, wherein the GR polypeptide comprises one of SEQ ID NO: 6 and SEQ ID NO: 8.

37. A crystallized GR ligand binding domain polypeptide produced by the method of claim 30.

38. A method for identifying a GR modulator, the method comprising:

(a) providing atomic coordinates of a GR polypeptide complex comprising an expanded binding pocket to a computerized modeling system;and

(b) modeling a ligand that fits spatially into the large pocket volume of the GR polypeptide complex to thereby identify a GR modulator.

39. The method of claim 38, wherein the polypeptide complex further comprises a co-activator and fluticasone propionate.

40. The method of claim 39, wherein the co-activator peptide is a TIF2 peptide.

41. The method of claim 40, wherein the TIF2 peptide comprises the sequence of SEQ ID NO: 9.

42. The method of claim 38, wherein the GR polypeptide comprises one of SEQ ID NO: 6 and SEQ ID NO: 8.

43. The method of claim 38, wherein the ligand is a non-steroid compound.

44. The method of claim 38, wherein the atomic coordinates comprise one of the atomic coordinates shown in Table 2 and a subset of the atomic coordinates shown in Table 2.

45. The method of claim 38, wherein the method further comprises identifying in an assay for GR-mediated activity a modeled ligand that increases or decreases the activity of the GR.

46. A method of designing a modulator that selectively modulates the activity of a GRα polypeptide comprising an expanded binding pocket, the method comprising:

(a) providing a crystalline form of a GRα polypeptide complex comprising an expanded binding pocket;

(b) determining the three-dimensional structure of the crystalline form of the GRα ligand binding domain polypeptide; and

(c) synthesizing a modulator based on the three-dimensional structure of the crystalline form of the GRα ligand binding domain polypeptide, whereby a modulator that selectively modulates the activity of a GRα polypeptide comprising an expanded binding pocket is designed.

47. The method of claim 46, wherein the GRα polypeptide complex further comprises a co-activator peptide and fluticasone propionate

48. The method of claim 46, wherein the co-activator peptide is a TIF2 peptide.

49. The method of claim 48, wherein the TIF2 peptide comprises the sequence of SEQ ID NO: 9.

50. The method of claim 46, wherein the GRα ligand binding domain comprises one of SEQ ID NO: 6 and SEQ ID NO: 8.

51. The method of claim 46, wherein the method further comprises contacting a GRα polypeptide with the potential modulator; and assaying the GRα polypeptide for binding of the potential modulator, for a change in activity of the GRα polypeptide, or both.

52. The method of claim 46, wherein the crystalline form is a hexagonal form.

53. The method of claim 46, wherein the crystalline form is such that the three-dimensional structure of the crystallized GRα polypeptide can be determined to a resolution of about 2.6 Å or better.

54. The method of claim 46, wherein the three-dimensional structure of the crystalline form of the GRα polypeptide complex is described by one of the atomic coordinates shown in Table 2 and a subset of the atomic coordinates shown in Table 2.

55. A method of forming a homology model of an NR, the method comprising:

(a) providing a template amino acid sequence comprising a GR polypeptide comprising an expanded binding pocket;

(b) providing a target NR amino acid sequence;

(c) aligning the target sequence and the template sequence to form a homology model.

56. The method of claim 55, wherein the GR polypeptide is in complex with a co-activator and fluticasone propionate.

57. The method of claim 56, wherein the co-activator peptide is a TIF2 peptide.

58. The method of claim 57, wherein the TIF2 peptide comprises the sequence of SEQ ID NO: 9.

59. The method of claim 55, wherein the GR polypeptide comprises one of SEQ ID NO: 6 and SEQ ID NO: 8.

60. The method of claim 55, further comprising assigning structural coordinates to the homology model.

61. The method of claim 55, wherein the NR is selected from the group consisting of AR, PR, ER, GR and MR.

62. The method of claim 55, wherein the template amino acid sequence comprises one of the atomic coordinates of Table 2 and a subset of the coordinates of Table 2.

63. The method of claim 55, wherein the template amino acid sequence comprises spatial coordinates characterizing an AF2 helix located in an active position, and wherein the spatial coordinates further characterize atoms in residues Met560, Met639, Gln642, Cys643, Met646, and Tyr735 that have shifted from their positions in a GR/Dex structure, characterized by the atomic structural coordinates of Table 3, by one of a heavy-atom RMS deviation of at least about 0.50 angstroms and by a backbone heavy-atom RMS deviation of at least about 0.35 angstroms.

64. The method of claim 55, wherein the template amino acid sequence comprises spatial coordinates characterizing an AF2 helix located in an active position, and wherein the spatial coordinates further characterize atoms in residues Met560, Met639, Gln642, Cys643, Met646, and Tyr735 that

have shifted from their positions in a GR/Dex structure, characterized by the atomic structural coordinates of Table 3, so as to increase the volume of a binding pocket by at least about 5%, compared with a GR/Dex structure characterized by the atomic structural coordiates of Table 3.

65. The method of claim 55, wherein the template amino acid sequence comprises spatial coordinates characterizing an AF2 helix located in an active position, and wherein the spatial coordinates further characterize atoms in and around a ligand binding site that have shifted from their positions in a GR/Dex structure, characterized by the atomic structural coordinates of Table 3, so as to accommodate, without atomic overlap, a steroidal ligand with C17-α substituents comprising 2-20 atoms.

66. The method of claim 55, wherein the template amino acid sequence comprises spatial coordinates characterizing an AF2 helix located in an active position, and wherein the spatial coordinates further characterize atoms in and around a ligand binding site that have shifted from their positions in a GR/Dex structure, characterized by the atomic coordinates of Table 3, so as to accommodate, without atomic overlap, a non-steroidal ligand.

67. The method of claim 55, wherein the template amino acid sequence comprises spatial coordinates characterize an AF2 helix located in an active position, and wherein the spatial coordinates further characterize atoms in and around a ligand binding site that have shifted from their positions in a GR/Dex structure, characterized by the atomic coordinates of Table 3, such that fluticasone propionate can be docked into a binding site with a favorable binding energy and wherein all atoms in the polypeptide are held fixed.

68. The method of claim 55, wherein the template amino acid sequence comprises spatial coordinates characterizing an AF2 helix is located in an active position, and wherein the spatial coordinates further

characterize atoms in and around the ligand binding site that have shifted from their positions in a GR/Dex structure, characterized by the atomic coordinates of Table 3, such that a non-steroidal GR ligand can be docked into the binding site with a favorable binding energy, as computed with molecular modeling software, and wherein all atoms in the polypeptide are held fixed.

69. A homology model formed by the method of claim 55.

70. A method of designing a modulator of a nuclear receptor, the method comprising:

(a) designing a potential modulator of a nuclear receptor that will make interactions with amino acids in the ligand binding site of the nuclear receptor based upon atomic structure coordinates of a NR polypeptide complex comprising an expanded binding pocket;

(b) synthesizing the modulator; and

(c) determining whether the potential modulator modulates the activity of the nuclear receptor, whereby a modulator of a nuclear receptor is designed.

71. The method of claim 70, wherein the potential modulator is a non-steroidal compound.

72. The method of claim 70, wherein the potential modulator is a steroid compound.

73. The method of claim 70, wherein the NR polypeptide complex further comprises a co-activator peptide and fluticasone propionate

74. The method of claim 70, wherein the NR polypeptide complex comprises a GR polypeptide.

75. The method of claim 74, wherein the GR ligand binding domain polypeptide comprises one of SEQ ID NO: 8 and SEQ ID NO: 10.

76. The method of claim 73, wherein the co-activator peptide is a TIF2 peptide.

77. The method of claim 76, wherein the TIF2 peptide comprises the sequence of SEQ ID NO: 9.

78. The method of claim 70, wherein the NR polypeptide is selected from the group consisting of AR, PR, ER, GR and MR.

79. The method of claim 70, wherein the atomic structure coordinates comprise one of the coordinates of Table 2 and a subset of the coordinates of Table 2.

80. A method of modeling an interaction between an NR and a non-steroid ligand, the method comprising:

(a) providing a homology model of a target NR generated using a crystalline GR polypeptide complex comprising an expanded binding pocket;

(b) providing atomic coordinates of a non-steroid ligand; and

(c) docking the non-steroid ligand with the homology model to form a NR/ligand model.

81. The method of claim 80, wherein the complex further comprises a co-activator and fluticasone propionate.

82. The method of claim 81, wherein the co-activator peptide is a TIF2 peptide.

83. The method of claim 82, wherein the TIF2 peptide comprises the sequence of SEQ ID NO: 9.

84. The method of claim 80, wherein the GR comprises one of SEQ ID NO: 6 and SEQ ID NO: 8.

85. The method of claim 80, wherein the NR is selected from the group consisting of AR, PR, ER, GR and MR.

86. The method of claim 80, wherein the homology model comprises one of the atomic coordinates of Tables 2-11 and a subset of the coordinates of Tables 2-11.

87 The method of claim 80, further comprising optimizing the geometry of the NR/ligand model.

88. A method of designing a non-steroid modulator of a target NR using a homology model, the method comprising:

(a) modeling an interaction between a target NR and a non-steroid ligand using a homology model generated using a crystalline GR polypeptide complex comprising an expanded binding pocket;

(b) evaluating the interaction between the target NR and the non-steroid ligand to determine a first binding efficiency;

(c) modifying the structure of the non-steroid ligand to form a modified ligand;

(d) modeling an interaction between the modified ligand and the target NR;

(e) evaluating the interaction between the target NR and the modified ligand to determine a second binding efficiency; and

(f) repeating steps (c)-(e) a desired number of times if the second binding efficiency is less than the first binding efficiency .

89. The method of claim 88, wherein the complex further comprises a co-activator and fluticasone propionate.

90. The method of claim 89, wherein the co-activator peptide is a TIF2 peptide.

91. The method of claim 90, wherein the TIF2 peptide comprises the sequence of SEQ ID NO: 9.

92. The method of claim 88, wherein the GR comprises one of SEQ ID NO: 6 and SEQ ID NO: 8.

93. The method of claim 88, wherein the target NR is selected from the group consisting of AR, PR, ER, GR and MR.

94. The method of claim 88, wherein the homology model comprises one of the atomic coordinates of Tables 2-11 and a subset of the coordinates of Tables 2-11.

95. A data structure embodied in a computer-readable medium, the data structure comprising: a first data field containing data representing spatial coordinates of an NR LBD comprising an expanded binding pocket, wherein the first data field is derived by combining at least a part of a second data field with at least a part of a third data field, and wherein

(a) the second data field contains data representing spatial coordinates of the atoms comprising a GR LBD comprising an expanded binding pocket in complex with a ligand; and

(b) the third data field contains data representing spatial coordinates of the atoms comprising a NR LBD.

96. The data structure of claim 95, wherein the data of the third data field comprises data selected from the data embodied in one of Table 3, Table 8, Table 9 and Table 10.

97. The data structure of claim 95, wherein the NR is selected from the group consisting of AR, MR, PR, ER and GR.

98. The data structure of claim 95, wherein the ligand is selected from the group consisting of bicalutamide and RWJ-60130.

99. The data structure of claim 95, wherein the GR is in further complex with a co-activator peptide.

100. The data structure of claim 99, wherein the co-activator peptide is a TIF2 peptide.

101. The data structure of claim 95, wherein the first data field comprises spatial coordinates describing a ligand in complex with the NR LBD.

102. The data structure of claim 95, wherein the ligand of the second data field is selected from the group consisting of bicalutamide and RWJ-60130.

103. The data structure of claim 95, wherein the spatial coordinates of the second data field characterize an AF2 helix located in an active position, and wherein the spatial coordinates further characterize atoms in residues Met560, Met639, Gln642, Cys643, Met646, and Tyr735 that have shifted from their positions in a GR/Dex structure, characterized by the atomic structural coordinates of Table 3, by one of a heavy-atom RMS deviation of at least about 0.50 angstroms and by a backbone heavy-atom RMS deviation of at least about 0.35 angstroms.

104. The data structure of claim 95, wherein the spatial coordinates of the second data field characterize an AF2 helix located in an active position, and wherein the spatial coordinates further characterize atoms in residues Met560, Met639, Gln642, Cys643, Met646, and Tyr735 that have shifted from their positions in a GR/Dex structure, characterized by the atomic structural coordinates of Table 3, so as to increase the volume of a binding pocket by at least about 5%, compared with a GR/Dex structure characterized by the atomic structural coordiates of Table 3.

105. The data structure of claim 95, wherein the spatial coordinates of the second data field characterize an AF2 helix located in an active position, and wherein the spatial coordinates further characterize atoms in and around a ligand binding site that have shifted from their positions in a GR/Dex structure, characterized by the atomic structural coordinates of Table 3, so as to accommodate, without atomic overlap, a steroidal ligand with C17-α substituents comprising 2-20 atoms.

106. The data structure of claim 95, wherein the spatial coordinates of the second data field characterize an AF2 helix located in an active position, and wherein the spatial coordinates further characterize atoms in and around a ligand binding site that have shifted from their positions in a GR/Dex structure, characterized by the atomic coordinates of Table 3, so as to accommodate, without atomic overlap, a non-steroidal ligand.

107. The data structure of claim 95, wherein the spatial coordinates of the second data field characterize an AF2 helix located in an active position, and wherein the spatial coordinates further characterize atoms in and around a ligand binding site that have shifted from their positions in a GR/Dex structure, characterized by the atomic coordinates of Table 3, such that fluticasone propionate can be docked into a binding site with a favorable binding energy and wherein all atoms in the polypeptide are held fixed.

108. The data structure of claim 95, wherein the spatial coordinates of the second data field characterize the AF2 helix is located in an active position, and wherein the spatial coordinates further characterize atoms in and around a ligand binding site that have shifted from their positions in a GR/Dex structure, characterized by the atomic coordinates of Table 3, such that a non-steroidal GR ligand can be docked into the binding site with a favorable binding energy, as computed with molecular modeling software, and wherein all atoms in the polypeptide are held fixed.

109. A method for designing a homology model of the ligand binding domain of an NR wherein the homology model may be displayed as a three-dimensional image, the method comprising:

(a) providing an amino acid sequence and an crystallographic structure of the ligand binding domain of a GRα polypeptide,

(b) modifying said crystallographic structure to take account of differences between the amino acid configuration of the ligand binding domains of the NR on the one hand and the GRα polypeptide on the other hand,

(c) verifying the accuracy of the homology model by comparing it with experimentally-determined NR protein and ligand properties, and if required, modifying the homology model for greater consistency with those binding properties.

110. A computational method of iteratively generating a homology model of the ligand binding domain of an NR, wherein the homology model is capable of being displayed as a three-dimensional image, the method comprising:

(a) entering into a computer a machine readable representation of an amino acid sequence of a ligand binding domain of a target NR polypeptide and a machine readable representation of a crystallographic structure of a ligand binding domain of a GRα polypeptide;

(b) identifying a difference between an amino acid configuration of a ligand binding domain of a target NR and a GRα polypeptide;

(c) modifying the machine readable representation of the crystallographic structure based on a difference identified in step (b) to thereby form a modified crystallographic structure;

(d) comparing the modified crystallographic structure with an experimentally-determined property of one of the target NR and a ligand of the target NR; and

(e) repeating steps (b) and (d) a desired number of times.

111. A homology model of the ligand binding domain of an NR produced by a method according to claim 109.

112. A homology model of the ligand binding domain of an NR produced by a method according to claim 110.